CHEMICAL ANALYSIS OF HUMAN SKIN EMANATIONS: COMPARISON OF VOLATILES FROM HUMANS THAT DIFFER IN ATTRACTION OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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ABSTRACT. Host odors are believed to play a major role in the location of blood meals by female mosquitoes. Previous work has shown that female *Aedes aegypti* (L.) are attracted to a residuum of skin emanations deposited on glass. The attraction of mosquitoes to handled or rubbed glass varies from person to person and from day to day. This variation indicates that mosquito behavior varies over time and that a relative difference exists in the ability of people over time to attract mosquitoes. Volatiles desorbed from glass handled by 2 human subjects that differed markedly in their attraction of *Ae. aegypti* were examined for differences in compound abundances. The attractive emanations, once deposited onto glass, are known to have a finite lifetime; therefore, compounds that decreased substantially during aging of handled glass also were noted. A study was conducted on the variations in compounds present from a single subject, which were recorded over a 5-day period. Emanations from the subject were transferred to glass, then thermally desorbed from the glass, and compounds present were compared on the 2 consecutive days that showed the largest difference in attraction. Some of the candidate attractants identified by these studies were screened in an olfactometer. A few of these compounds were found to be weak attractants for *Ae. aegypti.*

KEY WORDS Human emanations, attractants, kairomones, mosquitoes, *Aedes aegypti*

INTRODUCTION

Mosquitoes respond to human emanations with varying degrees of attraction (Schreck et al. 1990). The attraction to a host depends not only upon factors related to mosquito behavior, but also on the emanations produced by the host (Rahm 1957, Skinner et al. 1965b, Khan et al. 1967, Bar-Zeev et al. 1977, Schreck et al. 1981, Takken 1991, Eiras and Jepson 1994, Knols and Meijerink 1997, Knols et al. 1997). These emanations vary between individuals and vary over time for a single person (Khan et al. 1969, 1971), influenced partly by bacteria present on the skin, diet, exercise (Khan et al. 1969), and possibly other factors, such as pregnancy (Lindsay et al. 2000). The relative differences of components in emanations are suspected to be the reason why some human hosts are more attractive than others to *Aedes aegypti* (L.) (Schreck et al. 1990).

The work herein consists of studies that compare emanations from different humans, and variation of emanations from a single human on consecutive days for which the difference in attraction in laboratory bioassays was greatest. The variability in attraction has been reported to be a function of compounds in the residuum left on handled glass (Schreck et al. 1981). Handled glass collects a large number of compounds, many of which are skin lipids. At least some of these compounds transferred to glass are attractants for *Ae. aegypti*. Although all of the components that may be used in location of hosts are not necessarily transferred onto and off of glass, this is an interesting system for chemical examination because at least some of the important components involved in attraction are present. A well-known attractant present in the residuum is L-lactic acid (Acree et al. 1968), but this compound alone does not explain the high level of attraction to handled glass. Therefore, other chemical attractants necessary for optimal attraction are still unidentified.

The 2 subjects used for the comparison between individuals were selected based upon their relative attraction of *Ae. aegypti* in previous olfactometer bioassays (Schreck et al. 1990). One subject (A) routinely elicited high levels of mosquito attraction compared to another subject (B), who attracted fewer mosquitoes. A 3rd subject (C), in the middle range of mosquito attraction from the same study, was selected for experiments that focused upon the day-to-day variation in attraction. Use of a single subject minimizes chemical differences in the matrix on the skin, which are more diverse when different people are compared. Thus, chemical information of a single subject combined with biological information in the form of attraction efficiency can potentially provide valuable information about which volatile skin emanations are involved in location of hosts by mosquitoes. Emanations were collected with glass beads to transfer the sample from the skin (Bar-Zeev et al. 1977, Bernier et al. 1999); the majority of volatiles desorbed from handled beads have been identified previously (Bernier et al. 2000). Additionally, relative attraction of *Ae. aegypti* to handled beads is known to decrease over

time and handled beads are no longer attractive after 6 h (Schreck et al. 1981). Thus, volatile compounds that decreased in relative abundance over a period of 6–10 h also were examined by gas chromatography–mass spectrometry (GC-MS).

MATERIALS AND METHODS

Comparisons of beads from 2 human subjects and examination of aged beads: A series of experiments was conducted to compare emanations desorbed off 2.9-mm-diameter glass beads handled by 2 volunteers. Volunteer subject B in previous biological assays via an olfactometer consistently provided relatively low attraction of mosquitoes to handled glass (ca. 37%) and subject A provided the highest attraction percentage on a consistent basis (ca. 72%) (Schreck et al. 1990). Samples were analyzed in sets of 2 similar analyses, each involving beads handled by the 2 different subjects. In the morning session, 5 blank beads were analyzed before use. After arrival (at approximately 0800 h local time), subjects washed their hands with laboratory soap, dried them for 2 min, and 10–12 beads were handled for 15 min by the 1st subject. A chosen number of these beads, from 2 to 5 beads, were analyzed immediately after handling. The number used remained consistent throughout the day. The remainder of the rubbed beads were placed in a 0.25-in.-diameter glass tube at room temperature and open to ambient air. Fifteen minutes before the completion of the 1st analysis, the 2nd subject handled 10–12 beads. Between 2 and 5 of these beads, consistent with the previous analysis, were sampled immediately; the remainder were stored in a 2nd tube under the same conditions as previously described. The morning session involved either pulsed positive-ion negative-ion chemical ionization (PPINICI) (Hunt et al. 1976), or electron ionization (EI) as the ionization mode for GC-MS analysis. The mode chosen to analyze at the beginning of the day was alternated for each day for the 2-wk duration of the experiment to eliminate any possibility of problems resulting from degradation of column efficiency due to high-boiling components accumulating in the column from morning sessions.

The TSQ70 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) was switched over to the complementary mode for the afternoon session, that is, if PPINICI data were acquired in the morning session, EI data were acquired in the afternoon session (approximately 1300 h local time). An analysis of 5 blank beads was performed before the return of the subjects. The subjects repeated hand-washing and handling procedures. The beads were sampled and stored as described above for the morning session. Thus, the morning and afternoon sessions produced 4 data files, consisting of a PPINICI and EI analysis for each of the 2 subjects. The analyses were repeated in the evening, with the same ionization mode as used in the afternoon session, but with beads that had been stored, as described above, after being handled in the morning session. This allowed a 6- to 8-h gap between handling and GC-MS analysis. After each sample from the morning session was analyzed, the beads stored from the afternoon session were analyzed under the complementary ionization mode, producing another 4 data files. This procedure was conducted twice (2 different days) for each GC column (HP5 and HP-FFAP, Hewlett-Packard, Atlanta, GA). The GC parameters were dependent upon the GC column employed, as discussed in detail previously (Bernier et al. 2000). The mass spectrometer operation in EI and PPINCI modes was similar to that described previously (Bernier et al. 1999, 2000).

Comparison of a single human subject on consecutive days: Emanations were collected and analyzed daily from a single subject for 5 consecutive days. The collection process was similar to that for the study described for the comparison of subjects, except as follows. In this case, subject C served as the volunteer. On each of the 5 days, a blank was recorded in EI mode before analysis. The subject washed his hands with laboratory soap, dried them for 2 min, then handled 10 glass beads for 15 min. Three beads were used for each analysis. The remaining beads were stored for analysis with the complementary ionization mode. The cryofocusing stage for the 1st handled-bead analysis was initiated at approximately 0840 h local time each day. Upon completion of this analysis, the mass spectrometer ion volume was switched to allow for PPINICI. A blank was acquired and 3 beads were analyzed. The time delay between handling and analysis of the beads in this mode was approximately 2 h.

Bioassays of a glass petri dish handled by subject C were conducted approximately 1 h after sample collection for GC-MS analysis. These experiments were conducted with a triple-cage dual-port olfactometer (Posey et al. 1998). A petri dish was handled for 15 min before insertion into a port of the olfactometer. Testing occurred at approximately 1000 h local time each day with 75 (6- to 8-day-old) female *Ae. aegypti,* which were placed into the olfactometer cages 1 h before testing. The mosquitoes were chosen by the method of Posey and Schreck (1981). The relative humidity (60 ± 2%), air temperature (27 ± 1°C), and air flow (28 ± 1 cm/sec) were controlled throughout each analysis. Over the 5-day period, none of the 75 mosquitoes tested each day was collected in the control port. The lowest collection percentage (12%) to emanations from a handled dish occurred on the 1st day (Monday). The remainder of the week, from Tuesday through Friday, yielded collection percentages of 24, 21, 27, and 20%, respectively.

The analysis of handled beads was conducted at a lower GC oven temperature ramp rate than used for comparison of emanations from different subjects to provide additional resolution for coeluting
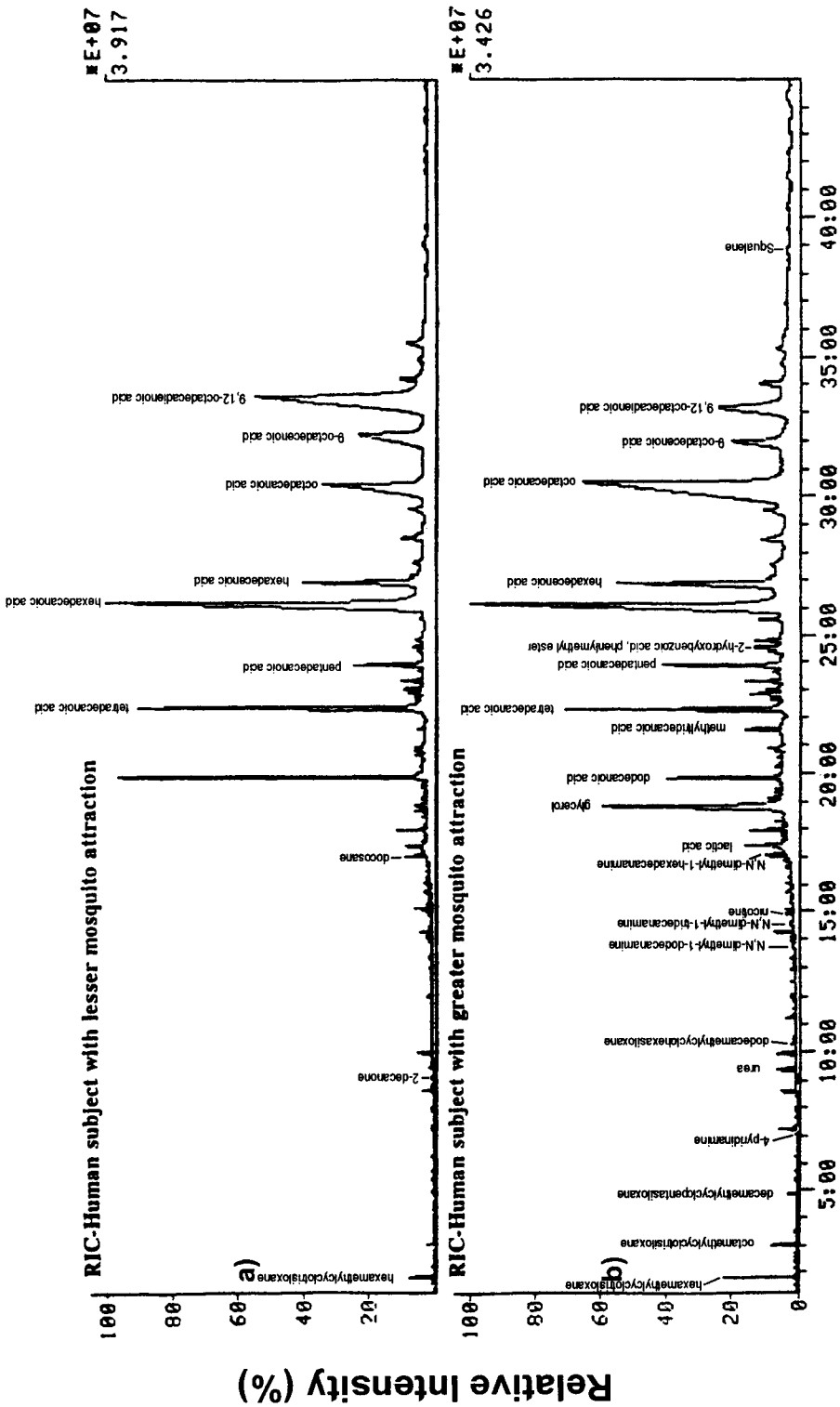
Fig. 1. Comparison of reconstructed ion chromatograms from glass beads handled by individuals who differed markedly in their attraction of *Aedes aegypti*. The chromatograms were acquired in electron ionization mode and separation was effected with the polar HP-FFAP column.

peaks detected in previous studies with a steeper temperature ramp (4 additional compounds were identified that previously had not been found). The GC oven ramp consisted of an initial 1.0-min hold at 40°C, a 26.7-min ramp at 6°C/min up to 200°C, and then held at 200°C for 12.3 min. The transfer line was concurrently ramped from 50°C up to 210°C at 10°C/min for 16.0 min, and held for 24.0 min at 210°C. Gas chromatographic separation was effected by a 25-m × 0.20-mm inner diameter HP5 fused silica open tubular column (film thickness = 0.33 μm).

The mass spectrometer operation in EI and PPINCI modes was similar to that described previously (Bernier et al. 1999, 2000). Methane reagent gas at 1,680–1,700 mTorr (indicated) pressure was employed for PPINICI analyses. The 3rd quadrupole was scanned at 0.5 sec per scan for both analysis modes. The conversion dynode was set at −5 kV for positive-ion CI and EI, and +5 kV for negative-ion CI. The electron multiplier was set to −1,000 V for EI experiments and −1,100 V for CI experiments. Before the analysis of blanks, the instrument was tuned with perfluorotributylamine.

Bioassay screening of candidate attractants: Screening of compounds was conducted by deposition of 50–500 μl for liquids, or 1–20 mg for solids, of the undiluted standard into a 60-mm petri dish. All compounds were purchased from either Sigma-Aldrich (Milwaukee, WI), Fluka (Milwaukee, WI), or Lancaster Synthesis (Windham, NH). The sample was bioassayed immediately after deposition and tested against a control consisting of an untreated 60-mm petri dish. Bioassays were conducted at 0830, 1100, and 1300 h local time. The procedures followed were otherwise identical to that described above for beads handled by subject C.

RESULTS AND DISCUSSION

Comparison of emanations from 2 different subjects

The reconstructed ion chromatogram of the beads handled by the less attractive subject contained, for the most part, the same peaks as those of the more attractive subject, as can be seen in the comparison of typical chromatograms for the 2 subjects (Fig. 1), although significant differences in the abundances of compounds are evident. The general pattern of carboxylic acids remained fairly consistent, as can be seen when compared to those published previously (Bernier et al. 1999, 2000).

Peak heights were measured and normalized to the peak height of dodecanoic acid because this peak was present in all analyses and was relatively consistent in abundance. The normalized peak height ratios of component peaks were then compared to determine specific compounds that were present in greater abundance for 1 subject compared to the other. The abundances of components present on the glass beads analyzed immediately after handling were also compared to those detected when analyzed 6–8 h later. The results of these comparative studies are presented in Table 1.

Forty-five compounds were increased in relative abundance in the host that was more attractive to mosquitoes. The qualitative terms "substantially" or "slightly" employed in the table merit some explanation. Because the relative abundances of components present in each sample fluctuate over time and therefore in the analyses, a clear indication of a single compound being responsible for the attraction is not observed. For example, lactic acid was found to vary from 5 times greater abundance (in subject A, the host with greater mosquito attraction capability, compared to B) to 1.3 times greater abundance in subject B compared to subject A. This may partially explain the attraction of *Ae. aegypti* to this host as well as fluctuations in mosquito attraction. In these same analyses, methyltetradecanoic acid remained fairly consistent, at 1.9–2.2 times greater relative abundance in the more attractive host. Therefore, the tables do not reflect an average value, but represent data describing what was observed in the majority of replicate analyses. However, the variation of component abundances from a single host should be considered when examining the results presented here. The listed compounds are likely candidates for attractants. Some of these have been tested via laboratory bioassays with an olfactometer; the results of the preliminary screening are presented as footnotes in Table 1.

The abundance of the lactic acid peak (17.3 min) in Fig. 1 fluctuated considerably from day to day and from person to person. This hydroxyacid is a primary component necessary for attraction of *Ae. aegypti* (Geier et al. 1999). Enzymatic removal of this compound from attractive skin washings results in a mixture that no longer is attractive to *Ae. aegypti* (Geier et al. 1996).

The glycerol peak (18.8 min) was present in much greater abundance in the sample from subject A, the host that was more attractive to *Ae. aegypti*. This was due to residual glycerol on the hands of the subject from the use of Sta-Sof-Fro™ hair and scalp spray (M&N Products, Smyrna, GA; Bernier et al. 2000). Glycerol was screened by bioassay in the olfactometer and neither activated nor attracted *Ae. aegypti* (Table 1).

The presence of 4-hexen-1-ol and 1-hepten-3-ol are of particular interest because of the structural similarity of these compounds to 1-octen-3-ol. The compound 1-octen-3-ol has been identified in human sweat (Cork and Park 1996) and attracts some species of mosquitoes (Takken and Kline 1989). If mosquito detection of chemical cues from hosts is class/structure dependent, then these seem to be good candidates for attractants; however, preliminary screening of these unsaturated alcohols did not elicit attraction of *Ae. aegypti*.

Other components of interest were the tertiary

Table 1. Comparison of compounds desorbed from glass beads handled by individuals that differ in their attraction of *Aedes aegypti*. Compounds that decreased markedly after an 8-h period from the more attractive host also are reported. This table is categorically divided into 5 sections. A substantial change reflects a relative difference or change by a factor of 5 or greater between hosts, or between host emanations and emanations detected after 8 h. A slight change refers to compounds with a relative difference of a factor range of 2–5.

Compound	Peak no.[1]
Compounds substantially increased in more attractive host	
Lactic acid[2]	117
Methyltridecanoic acid[3]	220
Pentadecanoic acid[4]	238
Hexadecenoic acid[4,5]	249
Octadecanoic acid[4]	269
4-hydroxy-3-methoxybenzoic acid	221
1-hepten-3-ol[4]	83
Glycerol[4]	131
Squalene[4]	299
Toluene[4]	51
N,N-dimethyl-1-dodecanamine	183
N,N-dimethyl-1-tridecanamine	199
N,N-dimethyl-1-tetradecanamine	216
N,N-dimethyl-1-hexadecanamine	244
N,N-dimethyl-1-octadecanamine	262
Methylhexadecanoic acid, methyl ester[3]	251
Octadecenoic acid, methyl ester[3]	261
2-hydroxybenzoic acid, phenylmethyl ester	242
Pentanedioic acid, ester[3]	273
Hexanedioic acid, mono(2-ethylhexyl ester)	280
Pyridine[4]	48
3-(1-methyl-2-pyrrolidinyl)pyridine (nicotine)	166
Oxazole	37
2,3-dihydro-3,5-methoxy-6-methyl-4*H*-pyran-4-one[3]	140
2-methylisothiazole	186
Butanone[2]	11
2-pentanone[2]	34
3-pentanone[2]	30
2-decanone[4]	146
Compounds slightly increased in the more attractive host	
Methyltetradecanoic acid[3]	233
Methylpentadecanoic acid[3]	246
Heptadecanoic acid[4]	258
4-hexen-1-ol[3,4]	56
2,5-bis(1,1-dimethylethyl)phenol (BHT)[6]	98
2-methylpropanal	72
Trimethyl-3-methylene hexadecane[3]	248
Docosane	271
2-butenoic acid, butyl ester[3]	38
4-hydroxybenzoic acid, propyl ester[3]	204
1-chlorotetradecane	202
4-pyridinamine[4]	66
2,3-dihydro-4-methylfuran	15
4H-pyran-4-one, substituted[3]	206
6-methyl-5-hepten-2-one[2]	107
Urea	25
Compounds substantially increased in the less attractive host	
Dodecanoic acid[4]	190
Cholesterol[4]	303
Methylpentanol	62
Heptane[4]	35
Methyliodide	9
1,3-butanediamine[3,4]	22
14-methylpentadecanoic acid, methyl ester	247
Compounds slightly increased in the less attractive host	
Decanoic acid[4]	161
Heptanal[4]	88

Table 1.   Continued.

Compound	Peak no.[1]
Nonanal[4]	135
2,4-nonadienal[3]	113
2-nonene[4]	85
Nonane[r]	87
Methylundecene[3]	124
Pentacosane[4]	297
Compounds substantially decreased after 8 h in the more attractive host	
Hexanedioic acid, mono(2-ethylhexyl ester)	280
2-methylpropanal	12
3-methylpentanal	49
N,N-dimethyl-1-dodecanamine	183
N,N-dimethyl-1-tridecanamine	199
N,N-dimethyl-1-tetradecanamine	216
N,N-dimethyl-1-hexadecanamine	244
N,N-dimethyl-1-octadecanamine	262
2-octene[4]	85
2-nonene[4]	55
Benzene[4]	29
Toluene[4]	51
Styrene[4]	86
Pyridine[4]	48
Oxazole	37
1H-indole[4]	191
Butanone[2]	11
2-pentanone[2]	34

[1] Peak numbers correspond to those listed in Bernier et al. (2000).
[2] Screened and found to be a weak attractant (10–30% of Ae. aegypti).
[3] Location of substitution or double bond is uncertain.
[4] Screened and found not to attract Ae. aegypti (0–10% relative attraction).
[5] Although 9-hexadecenoic acid was reported by Bernier et al. (2000) and screened here, the predominant unsaturated C_{16} acid for adults is 6-hexadecenoic acid (Stewart 1992).
[6] Present in background analyses.

amines discussed previously (Bernier et al. 2000). Although ammonia was not detected, it is a component of incubated sweat that is attractive to *Anopheles gambiae* sensu stricto (Braks and Takken 1999, Braks et al. 2000) and *Ae. aegypti* (Geier et al. 1999). The amines reported here were substantially more abundant in the more attractive host and found to decrease substantially after the sample aged for 8 h. These 2 criteria fit the profile of a candidate attractant. That is, compounds that are present in greater abundance in the more attractive host and that decrease markedly after 6 h or more (Schreck et al. 1981). The origin of these compounds remains a concern; it is unknown whether or not these compounds originate from humans or are deposited on the skin by exogenous sources. Pyridine was detected at higher levels on beads handled by the more attractive host and this compound did evaporate substantially from stored beads. A trace amount of this compound was found in incubated human sweat, which is attractive to *An. gambiae* (Meijerink et al. 2000); however, pyridine has not been reported as an attractant for this species, nor was it found to attract *Ae. aegypti* here (Table 1).

Several ketones (butanone, 2-pentanone, 3-pentanone, 2-decanone, and 6-methyl-5-hepten-2-one)

were more abundant on the host that attracted a higher percentage of mosquitoes in bioassays. Butanone and 2-pentanone evaporated rapidly from glass beads, and thus were examined as candidate attractants. These highly volatile ketones activated *Ae. aegypti* to flight in the olfactometer and were weak attractants. The compound 6-methyl-5-hepten-2-one has been observed previously in GC-MS studies (Labows et al. 1979, Bernier et al. 2000) and GC-MS with electroantennogram studies of human sweat components (Meijerink et al. 2000). Butanone has been evaluated as a mosquito attractant in the field in Florida (Kline et al. 1990, Kline and Mann 1998). Combined with carbon dioxide, butanone increased trap catches, but was much less efficient than 1-octen-3-ol combined with carbon dioxide. It is worthwhile to note that of the single compounds bioassayed in our work, a substantial number of the weak attractants were ketones. This class also contains acetone, which does activate and attract *Ae. aegypti,* as well as *An. gambiae* (Takken et al. 1997, Takken and Knols 1999).

Squalene, the precursor to cholesterol, has been reported as a long-distance tick attractant (Yoder et al. 1999), but squalene did not attract *Ae. aegypti* in our bioassays (Table 1). Cholesterol, also found in large amounts on human skin, did not produce
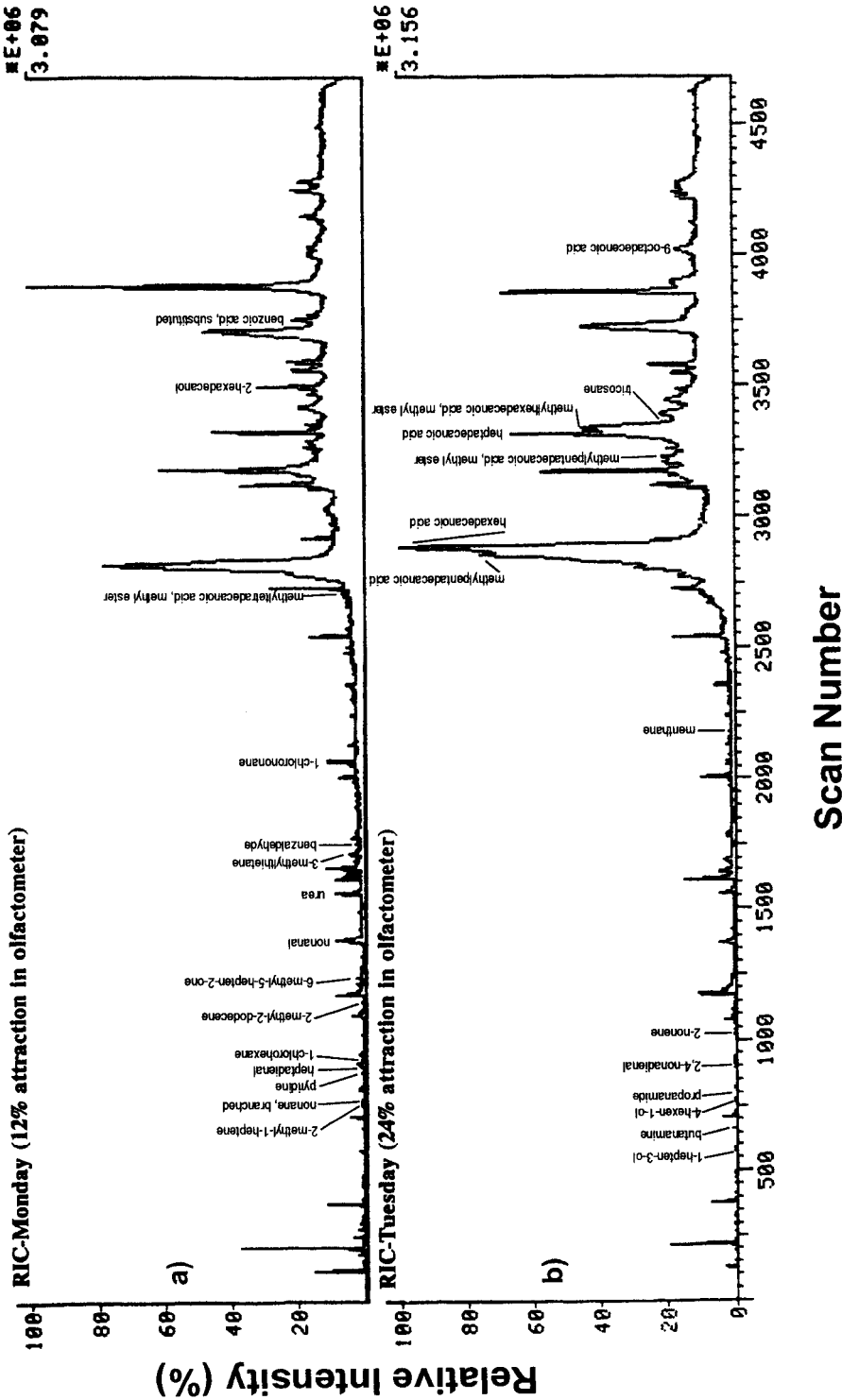
Fig. 2. Comparison of reconstructed ion chromatograms from glass beads handled by the same individual (subject C) on consecutive days, where a difference in attraction was noted between the consecutive days. The chromatograms were acquired in electron ionization mode and separation was effected with the polar HP-FFAP column.

Table 2. Comparison of emanations desorbed from glass beads handled on consecutive days by a single individual. Bioassays conducted on those days showed 12 and 24% attraction of mosquitoes (*Aedes aegypti*) in an olfactometer. An increase in the table reflects a compound found to be present in a greater relative amount on the 2nd day (24% attraction) with respect to the previous day (12% attraction). A substantial change reflects a relative difference by a factor of 5 or greater between days. A slight change refers to compounds with a relative difference of a factor range of 2–5.

Compound	Peak no.[1]
Compounds substantially increased on more attractive day	
Heptadecanoic acid[2]	258
Methylpentadecanoic acid[3]	246
Hexadecanoic acid[2]	250
1-hepten-3-ol[2]	83
Propanamide	128
Compounds slightly increased on more attractive day	
9-octadecenoic acid[2]	267
4-hexen-1-ol[2]	56
2,4-nonadienal	113
Butanamine[3,4]	—
2-nonene	85
Menthane[3]	130
Tricosane	274
13-methylpentadecanoic acid, methyl ester	235
Methylhexadecanoic acid, methyl ester[3]	251
Compounds substantially decreased on more attractive day	
Benzoic acid, substituted[3]	221
Heptadienal[3,4]	—
2-methyl-1-heptene	46
Nonane, branched[3,4]	—
1-chlorohexane	76
1-chlorononane	129
Pyridine[2]	48
Urea	25
Compounds slightly decreased on more attractive day	
2-hexadecanol[3]	245
Nonanal[2]	135
Benzaldehyde[2]	106
2-methyl-2-dodecene	145
Methyltridecanoic acid, ethyl ester[3,4]	—
6-methyl-5-hepten-2-one[5]	107
3-methylthietane	68

[1] Peak numbers correspond to those listed in Bernier et al. (2000).
[2] Screened and found not to attract *Ae. aegypti* (0–10% relative attraction).
[3] Location of substitution or double bond is uncertain.
[4] Unreported in previous publication (Bernier et al. 2000).
[5] Screened and found to be a weak attractant (10–30% of *Ae. aegypti*).

any distinct behavior in bioassays. The occurrence of nicotine on the skin of the more attractive host, compared to the less attractive host, is most likely due to the use of tobacco by the more attractive subject.

Carboxylic acids are the major constituents of human skin emanations (Nicolaides et al. 1970, Nicolaides 1974). Electroantennogram responses from *An. gambiae* were greater for acids of shorter chain lengths (Cork and Park 1996). Recently, short-chain fatty acids have been shown to be attractive for *Ae. aegypti* when combined with L-lactic acid (Bosch et al. 2000). The acids reported to differ in abundance between the human subjects examined in this work are much larger in chain length and

none of these acids that were tested by bioassay produced an attraction response with *Ae. aegypti*. However, the general pattern of carboxylic acids is similar among humans, yet unique when compared to that in other mammals (Nicolaides et al. 1968, Nicolaides 1974). The chromatograms and compounds present in the chromatograms are very similar for acids found under the toenail compared to those from Limburger cheese (Knols and Meijerink 1997, Knols et al. 1997). Furthermore, the chromatograms for volatiles from feet and Limburger cheese are very similar to those reported from human emanations of the hand and arm (Bernier et al. 1999, 2000). Many of the same carboxylic acids have been combined in a blend and tested for at-

traction of *An. gambiae* s.s. (Healy and Copland 2000). The compound 2-oxopentanoic acid elicited a landing response for this species; however, this compound has not yet been confirmed as a skin emanation present in sweat.

A number of reported alkanes, alkenes, and aldehydes (Ellin et al. 1974, Bernier et al. 2000) were more abundant on the host that was less attractive to *Ae. aegypti.* Although hydrocarbons contribute little to the overall attraction, the aldehydes tend to have an inhibitory effect when tested in combination with L-lactic acid (U. R. Bernier, unpublished data). Polar constituents in the lipid phase of skin washings have been reported as being repellent and this indicates that humans may have both attractive and repellent or inhibitory substances emanating from the skin (Skinner et al. 1965a, Maibach et al. 1970). The reduction in attraction is similar to that observed for the inclusion of undecanoic or tetradecanoic acids with the attractant mixture of Bosch (Bosch et al. 2000). This supports the hypothesis that mosquito attraction to a host is a complex combined effect of attraction, due to multiple odor components, and inhibition (repellency), which also is most likely due to numerous odor components.

Comparison of a single human subject on consecutive days

The 2nd set of experiments involved GC-MS analysis and bioassay of emanations from a single host collected once per day over a 5-day period. Correlation of the fluctuations in attractiveness of a single subject with changes in compound abundances was thought to have the greatest potential for identifying candidate attractants. The advantage of this comparison is that a single host provides a more stable and consistent matrix on the skin, with less chance of markedly different trace emanations. The drawback is that the subject's attraction to mosquitoes may not vary as greatly as it would for persons chosen at the extremes of attraction.

The range of attraction in the olfactometer for the single human subject C over the 5-day period was between 12 and 27%. The comparison of compound abundance employed only the 1st 2 days (12 vs. 24%) because the majority of components in the skin matrix are believed to change the least over consecutive days. Reconstructed ion chromatograms from samples analyzed on these 2 days are shown in Fig. 2.

As reported in Table 2, 14 compounds, including the unsaturated alcohols mentioned above, were increased on the more attractive day; 15 compounds were found to decrease. This discrepancy may reflect normal variation in abundances from day to day, as was seen throughout many of the analyses conducted for the previous case study.

Both 4-hexen-1-ol and 1-hepten-3-ol were present at increased relative abundance on the day the human subject was more attractive to mosquitoes.

Because of the differences observed in these alcohols here and in the previous study, these compounds were thought to be good candidates as attractants. However, as mentioned in the discussion of the previous study, neither of these compounds was found to attract *Ae. aegypti.* Only 1 compound, 6-methyl-5-hepten-2-one, from this study of a single subject elicited any attraction of mosquitoes in bioassays. However, this component was present in lower abundance on the day the subject was more attractive to *Ae. aegypti.*

In summary, the comparison of the same subject on consecutive days showed less overall variation (compared to the previous study with different subjects) of relative abundances of sample components present. Unfortunately, this comparison also complicates our ability to identify potential attractants. Important volatile compounds used by mosquitoes to locate suitable hosts may not have been detected here. Some human emanations did attract *Ae. aegypti* weakly, with attraction roughly on the same level as for L-lactic acid. On-going studies are examining these compounds in blends for possible synergism and microscale purge and trap GC-MS analyses are being used to identify the more volatile compounds that have not been detected by the GC-MS method employed here and to quantify the compounds that emanate from the skin.

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